Purpose - To determine the prevalence, anatomical location and severity of cartilage defects in the stifle (knee) within a population of adult ewes enrolled for research.
Methods - *Animals:* hind limbs (n= 60) from 30 adult ewes collected within 6 hours of euthanasia. *Gross anatomical observation:* the articular surfaces of the distal femur, proximal tibia and patella were examined by gross observation by two pairs of investigators. They scored the abnormalities of the hyaline cartilage using OARSI recommendations for ovine cartilage: score 0 for intact cartilage surface; score 1 for surface roughening; score 2 for deeper defects (fibrillation, fissures) not involving the subchondral bone; score 3 for small erosions down to subchondral bone (less than 5 mm diameter); score 4 for large erosions down to subchondral bone (more than 5 mm diameter). Scoring of articular surfaces was performed in 26 anatomic areas in each knee. Discrepancies were discussed between investigators and pairs till a consensus was reached. *Histopathology*: for each anatomical location where lesions had been identified, osteochondral slabs were obtained for histopathology. Samples were obtained in several regions with negative findings (score 0) for control. Conventional light microscopy [staining with Toluidine Blue and Safranin O) was performed to characterize structure of cartilage and bone, and confirm the classification of lesions performed by gross observation. *Scoring and grading*: the most severe lesion observed in each of the 26 anatomic regions was used to score the articular surface of that region. Grading of cartilage defects within each knee was obtained by the addition of the scores of all regions.
Results - There were 23 Texel and 7 Iles de France ewes, aged 3 to 11 years, weighting between 38 and 72 kg, euthanized between May and October 2011. There were anatomical locations where cartilage defects were significantly ($p < 0.05$) more frequently identified than in others: central third of the medial femoral condyle (MFC2), axial aspect of the central third of the medial tibial condyle (AxMTC2), axial aspect of the central third of the lateral tibial condyle (AxLTC2). Score 2 defects were present respectively in 25, 31 and 15 % of all stifles on MFC2, AxMTC2 and AxLTC2 while score 1 surface abnormalities were present in 36, 25 and 5 % respectively. There was no effect of side (left or right), weight or breed on the grade of cartilage defect in the knee. Though not reaching statistical significance, grade increased moderately with age in this population ($p = 0.08$ and b= 0.02).
Conclusions - This study demonstrated that score 2 cartilage defects can be significantly prevalent in stifles in a population of sheep enrolled for research. It indicates that, in Texel and Iles de France used for research, it is useful to assess cartilage status at baseline before including the animal in the experimentation, and to enroll, for each group of the trial, animals presenting either no cartilage defect or a same grade of defect. This should be assessed by non-invasive imaging modalities at baseline. Further research is required to evaluate the development of those lesions over time and compare imaging modalities in their ability to assess naturally occurring cartilage defects in the ovine stifle.

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CHARACTERISING NON-SURGICAL MURINE KNEE OSTEOARTHRITIS MODEL PRODUCED BY CONTROLLED FORCED RUNNING

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Purpose: To develop and characterize a nonsurgical murine knee osteoarthritis (OA) model where the mechanical load is applied by forced running on a mechanized rodent treadmill.
Methods: The mechanical loading was controlled by changing number of forced running periods and running time. In the time course study, mice (n = 4) ran for 15 min at 11 m/min three times a week (1 km/2 weeks), and both knees were retrieved after 2, 4, 6, and 8 weeks. In the dose-response study, mice (n = 4) ran for a brief (7.5 min) or longer (30 min) time at the same speed and frequency as noted above, and both knees were retrieved 2 weeks later. Changes in the joints were observed radiologically and histologically. Cartilage degeneration was quantified using the OARSI grading scale. Changes in types II and X collagen expression were examined by immunohistochemistry.
Results: Changes in the joint tissues, including meniscal ossification, osteophyte formation, cruciate ligament degeneration, and the OARSI score increased significantly in a time- and dose-dependent manner in proportion to the total load applied.
Immunohistochemistry showed reduced type II collagen expression and induction of type X collagen expression in the articular cartilage after forced running.
Conclusions: This murine knee OA model offers opportunities to study the effects of varying the loading on joint health and disease, and the interactions between genetics and mechanical influences on OA initiation and progression.

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EXPRESSION OF PPARα, β, AND γ IN THE HARTLEY GUINEA PIG MODEL OF PRIMARY OSTEOARTHRITIS

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Purpose: To investigate the expression of PPARα, β, and γ in cartilage over the course of osteoarthritis (OA) in the spontaneous Hartley guinea pig model.
Methods: Hartley guinea pigs were sacrificed at 2 (control group), 4, 8, and 12 (n = 6 per group) month-old of age. Cartilage was obtained from the central portion of the medial tibial plateau. Cartilage degradation was evaluated histologically using the Osteoarthritis Research Society International (OARSI) guidelines. The expression of PPARα, β and γ was analyzed by immunohistochemistry. The non-parametric Spearman test was used for the correlation analysis between the protein expression levels and histological scores.
Results. PPARα, β and γ, were detected in medial tibial plateaus from control animals. There was no significant changes in the levels of PPARα and PPARβ over the course of OA. In contrast, PPARγ expression decreased during the progression of OA. Correlation analysis revealed a negative correlation between PPARγ levels and histological score of OA.
Conclusion. Expression of PPARγ in cartilage decreased during the course of OA. These data suggest that loss of PPARγ expression in cartilage may contribute to the pathogenesis of OA.

Biomarkers

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XANTHINE OXIDASE RESPONSE IN ACUTE JOINT INJURY

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Purpose: Xanthine oxioreductase (XOR) is a widely distributed molybdoflavoenzyme enzyme that catalyzes the oxidation of hypoxanthine to xanthine and further catalyzes the oxidation of xanthine to uric acid (UA). XOR appears in two distinct interconvertible forms. XOR is constitutively expressed as a dehydrogenase but can be post-translationally modified by reversible thiol oxidation or irreversible proteolytic cleavage to its oxidase form (XO). Circulating XOR exist almost exclusively as the proteolytic generated XO. In the XO form, through the process of oxidizing purines to urate, it becomes a major source of reactive oxygen species (ROS) production. XO generated ROS such as superoxide and H2O2 are well known to cause damage to many cell types, including synoviocytes and chondrocytes. We hypothesized that there would be an increase in synovial fluid (SF) XO activity in acute joint injury with an attendant rise in ROS that could contribute to further joint damage. To our knowledge this is the first study of XO activity in acute joint injury.
Methods: Patients: 11 patients without osteoarthritis (OA), (ages 18-29, mean 23) were enrolled in a pilot intervention trial consisting of a single intra-articular knee injection of interleukin-1 receptor antagonist or saline following joint injury with anterior cruciate ligament tear. SF and serum were collected from 9 of these subjects at presentation to the clinic after

acute knee injury (mean 15 +/- 7 days) and at the follow-up visit for reconstructive surgery (mean 48 +/- 12 days) for a total of 18 samples. SF from 23 subjects with minimal knee OA (KL grade 0-1, ages 38-81, mean 64) was used as a non injury control group.

SF analysis: XO activity and protein carbonyl were measured using kits from Cayman Chemical (Ann Arbor, MI). UA was analyzed using HPLC. As indicators of collagen synthesis and degradation, procollagen II C-propeptide (CPII) and collagen type II telopeptide (CTxII) were measured by ELISA (Ibex, Montreal, Quebec, and IDS, Scottsdale, AZ respectively).

Statistical Analysis: Graphpad Prism software (San Diego, CA).

Results: The 18 samples from the 9 subjects in the acute injury cohort were treated as a single group for the purpose of this analysis because no effect was observed on any of the above analytes due to treatment group or differences in time course, nor were any trends seen between the two time points. SF XO activity was significantly higher (14X) than serum XO activity in the acute injury group and was significantly higher (5X) than control group SF XO activity (Figure 1). SF carbonyl concentration was significantly higher (2.5X) in the acute injury group than the control group (Figure 2a). Importantly, this indicator of ROS production showed a strong positive association with XO activity in the injury group (Figure 2b). SF UA was significantly lower (1.5X) in the acute injury group than the control group and there was a strong negative correlation of UA with XO activity and carbonyl formation (Figure 3). SF CPII also showed a negative correlation with XO activity while no association was found between SF XO and SF CTxII (Figure 4).

Conclusions: This study shows that SF XO activity is increased in acute joint injury and that this increase is strongly associated with an increase in the production of ROS as measured by protein carbonyl content. These data confirm that oxidative stress participates in the pathophysiology of joint damage following acute joint injury. While an increase in UA production might also have been expected, in fact, we observed a negative correlation between XO and UA indicating that any extra UA being produced is being consumed as an antioxidant. This is not surprising, since UA is known to be a potent antioxidant. This increased XO activity may also be contributing to a decrease in type II collagen production thus hindering the repair response of the acutely injured joint.

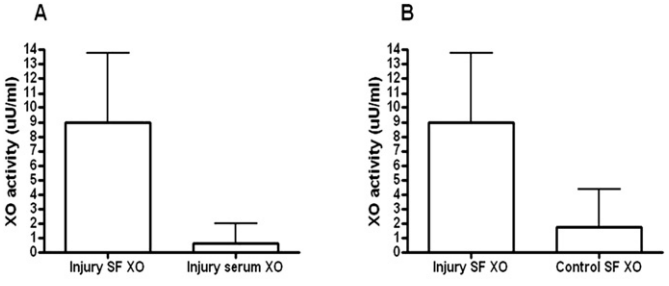

Figure 1. A) XO activity significantly higher in SF than serum in acute injury (p=0.0003) .
B) SF XO activity significantly higher in acute injury than control (p<0.0001).

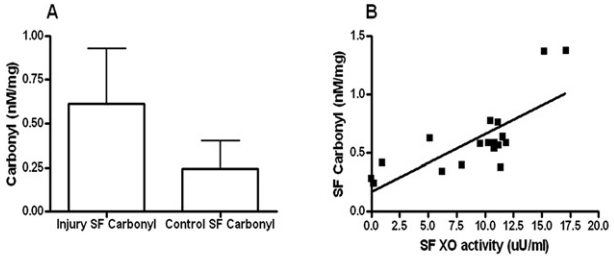

Figure 2. A) SF carbonyl significantly higher in acute injury than control (p<0.0001) .
B) SF XO positively correlated with SF carbonyl in acute injury (r^2=0.564, p=0.0003).

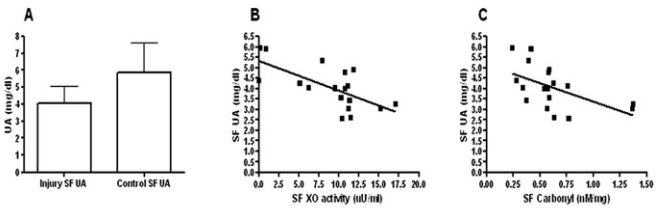

Figure 3. A) SF UA significantly lower in acute injury than control (p=0.0002) .
B) SF XO negatively correlated with SF UA in acute injury (r^2=0.453, p=0.002).
C) SF Carbonyl negatively correlated with SF UA in acute injury (r^2=0.284, p=0.02).

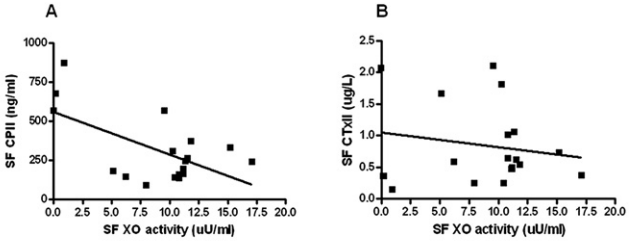

Figure 4. A) SF XO activity negatively correlated with SF CPII in acute injury (r^2=0.362, p=0.01) .
B) No association of SF XO activity with SF CTxII.

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CAN BONE SHAPE PREDICT WHO WILL HAVE THEIR KNEE REPLACED? - DATA FROM THE OAI

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Purpose: Total knee replacement (TKR) is an important clinical endpoint in osteoarthritis pathogenesis. Biomarkers capable of predicting TKR may be valuable markers for treatment decisions and treatment development, especially when testing DMOAD efficacy in clinical trials. The purpose of this study was to determine a)whether specific locations of bone shape curvature are associated with TKR compared to an age-gender-KL matched control group, and b)whether those differences predict TKR.

Methods: 4,796 participants from the Osteoarthritis Initiative (OAI) were studied. Participants with TKR between 12-48 months of follow-up, confirmed by radiography and/or review of hospital records, were selected. TKR knees were matched with control knees for radiographic stage (KL 0/1, 2, 3 and 4), sex and age +/- 5 years. Quantitative bone curvature measures were obtained from sagittal 3DWE DESS acquired at the time point prior to TKR (T0). CiPAS software (Qmetrics, Rochester, NY) was used to create bone curvature maps. Mean and standard deviation maps were computed and the location of statistical differences in curvature between cases and controls were found using statistical parametric mapping (SPM) analysis with point by point t-test analysis adjusted for false discovery. The relevance of the mean curvature at different knee regions of interest (Femur, Tibia, cMF, cLF, MT, LT and Trochlea) as potential diagnostic biomarkers was tested using the Wilcoxon signed rank test, and reported ROC analyses of curvature adjusted for age, gender, KL and height and ROC of an unconditional logistic model controlled for Gender, KL, Age, BMI, Pain and Curvature at T0.

Results: 127 knees of OAI participants with TKR had central X-ray readings, T0 MRI data and matched controls (76F, 46M, age 64y, BMI 30) were included. The false discovery-adjusted SPM analysis indicated that 4.4% of the femoral subchondral bone surface showed statistical differences towards flattening (RMS t=2.7, p=0.004) when comparing cases and matched controls. The largest affected area was the central medial femur representing only 2.7% of the subchondral bone surface. The same analysis